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**☐ 1:** PCR Methods Appl 1995 Jun;4(6):357-62

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Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.

Livak KJ, Flood SJ, Marmaro J, Giusti W, Deetz K.

Applied Biosystems Division, Foster City, California 94404, USA.

The 5' nuclease PCR assay detects the accumulation of specific PCR product by hybridization and cleavage of a double-labeled fluorogenic probe during the amplification reaction. The probe is an oligonucleotide with both a reporter fluorescent dye and a quencher dye attached. An increase in reporter fluorescence intensity indicates that the probe has hybridized to the target PCR product and has been cleaved by the 5'-->3' nucleolytic activity of Taq DNA polymerase. In this study, probes with the quencher dye attached to an internal nucleotide were compared with probes with the quencher dye attached to the 3'-end nucleotide. In all cases, the reporter dye was attached to the 5' end. All intact probes showed quenching of the reporter fluorescence. In general, probes with the quencher dye attached to the 3'-end nucleotide exhibited a larger signal in the 5' nuclease PCR assay than the internally labeled probes. It is proposed that the larger signal is caused by increased likelihood of cleavage by Taq DNA polymerase when the probe is hybridized to a template strand during PCR. Probes with the quencher dye attached to the 3'-end nucleotide also exhibited an increase in reporter fluorescence intensity when hybridized to a complementary strand. Thus, oligonucleotides with reporter and quencher dyes attached at opposite ends can be used as homogeneous hybridization probes.

PMID: 7580930 [PubMed - indexed for MEDLINE]

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     Rebek, Culius, Jr.
     The Scripps Research Institute, USA
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     FCT Int. Appl., 62 pp.
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     energy transfer labels and methods based
     thereon.
ΑU
     Mazarenko, Irina A. (1); Bhatnagar, Satish K.; Winn-Deen, Emily S.;
     Hohman, Robert J.
     (1) Gaithersburg, MD USA
     ASSIGNEE: Interden Company, Purchase, NY, USA
     US 6090552 July 18, 2000
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      (July 18, 2000) Vol. 1236, No. 3, pp. No Pagination. e-file.
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     Nazarenko, Irina A.; Bhatnagar, Satish K.; Winn-Deen, Emily S.; Hohman,
     Robert J.
     ASSIGNEE: Interger Company
     US 6117635 September 12, 2000
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     Mazarenko, I. A.; Bhatnagar, S. K.; Winn-Deen, E. S.; Hohman, R. J.
CS.
     Gaithersburg, Md. USA
     ASSIGNEE: ONCOR, INC.
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